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(54) Title: AEROSOLS CONTAINING NANOPARTICLE DISPERSIONS		
(57) Abstract There is disclosed an aerosol comprising droplets of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof. There is also disclosed a method for making the aerosol and methods for treatment and diagnosis using the aerosol.		

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AEROSOLS CONTAINING NANOPARTICLE DISPERSIONS
FIELD OF THE INVENTION

The present invention is directed to the field of nanoparticles and particularly in an aerosol form.

5 BACKGROUND OF THE INVENTION

Delivery of therapeutic agent to the respiratory tract is important for both local and systemic treatment of disease. With the conventional techniques, delivery of agents to the lung is extremely inefficient. Attempts to develop respirable aqueous suspensions of poorly soluble compounds have
10 been unsuccessful. Micronized therapeutic agents suspended in aqueous media are too large to be delivered by aerosolized aqueous droplets. With conventional processes, it is estimated that only about 10 to 20% of the agent reaches the lung. Specifically, there is loss to the device used to deliver the agent, loss to the mouth and throat and with exhalation. These losses lead to
15 variability in therapeutic agent levels and poor therapeutic control. In addition, deposition of the agent to the mouth and throat can lead to systemic absorption and undesirable side effects.

The efficiency of respiratory drug delivery is largely determined by the particle size distribution. Large particles (greater than 10 m) are prim-
20 arily deposited on the back of the throat. Greater than 60% of the particles with sizes between 1 and 10 m pass with the air stream into the upper bronchial region of the lung where most are deposited. With particles less than about 1 μ m, essentially all of the particles enter the lungs and pass into the peripheral alveolar region; however, about 70% are exhaled and therefore are lost.

25 In addition to deposition, the relative rate of absorption and rate of clearance of the therapeutic agent must be considered for determining the amount of therapeutic agent that reaches the site of action. Since 99.99% of the available area is located in the peripheral alveoli, rapid absorption can be realized with delivery of the particles to the periphery.
30 For clearance, there is also differences between the central and peripheral regions of the lung. The peripheral alveolar region does-not have ciliated cells but relies on macrophage engulfment for

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particle clearance. This much slower process can significantly extend the time during which the particles reside in the lung thereby enhancing the therapeutic or diagnostic effect. In contrast, particles deposited in the upper respiratory tract are rapidly cleared by mucociliary escalator. That is, the particles are trapped in the mucous blanket coating the lung surface and are transported to the throat. Hence, this material is either swallowed or removed by coughing.

While it has long been known that smaller droplets of an aerosol reach deeper into the respiratory system (Current Concepts in the Pharmaceutical Sciences: Dosage and Bioavailability, J. Swarbrick Ed., Lea and Febiger, Philadelphia, PA, 1973, pp. 97-148) these have largely been of theoretical interest. Simply knowing that smaller droplets of aerosol can be delivered deeper into the respiratory system does not solve the problem of incorporating sufficient therapeutic agent into the aerosol to be efficient, particularly where the therapeutic agent is only slightly soluble in the liquid for the aerosol.

Nanoparticles, described in U.S. Patent No. 5,145,684, are particles consisting of a poorly soluble therapeutic or diagnostic agent onto which are adsorbed a non-crosslinked surface modifier, and which have an average particle size of less than about 400 nanometers (nm). However, no mention is made of attempts to nebulize (aerosolize or atomize are equivalent terms for the purpose of this disclosure) these compositions and it is not apparent that nebulizing these composition would provide useful aerosols or that there would be any advantage for doing so.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided an aerosol comprising droplets of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof.

In another aspect of the invention, there is provided a method for forming an aerosol of a nanoparticle dispersion, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof, said method comprising the steps of:

- a) providing a suspension of said nanoparticles;
- b) nebulizing said suspension so as to form an aerosol.

In yet another aspect of the invention, there is provided a method of treating a mammal comprising the steps of:

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a) forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic agent particles having a surface modifier on the surface thereof;

b) administering said aerosol to the respiratory system of said
5 mammal.

In yet another embodiment, there is provided a method of diagnosing a mammal, said method comprising

a) forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble diagnostic imaging agent
10 particles having a surface modifier on the surface thereof;

b) administering said aerosol to the respiratory system of said mammal; and

c) imaging said imaging agent in said respiratory system.

15 DETAILED DESCRIPTION OF THE INVENTION

The compositions of the invention are aerosols. Aerosols can be defined for the present purpose as colloidal systems consisting of-very finely divided liquid droplets dispersed in and surrounded by a gas. The droplets in the aerosols typically have a size less than about 50 microns in diameter although
20 droplets of a much smaller size are possible.

The aerosols of the present invention are particularly useful in the treatment of respiratory related illnesses such as asthma, emphysema, respiratory distress syndrome, chronic bronchitis, cystic fibrosis and acquired immune deficiency syndrome including AIDS related pneumonia.

25 The aerosols of the invention are made by nebulizing the nanoparticle containing solution using a variety of known nebulizing techniques. Perhaps the simplest of systems is the "wo-phase" system which consists of a solution or a suspension of active ingredient, in the present case, a nanoparticle containing a therapeutic or diagnostic agent, in a liquid propellant. Both liquid
30 and vapor phases are present in a pressurized container and when a valve on the container is opened, liquid propellant containing the nanoparticle dispersion is released. Depending on the nature of the ingredients and the nature of the valve mechanism, a fine aerosol mist or aerosol wet spray is produced.

35 There are a variety of nebulisers that are available to produce the aerosols of the invention including small volume nebulizers. Compressor driven nebulizers incorporate jet technology and use compressed air to generate the

aersol. Commercially available devices are available from Healthdyne Technologies Inc; Invacare Inc.; Mountain Medical Equipment Inc.; Pari Respiratory Inc.; Mada Mediacal Inc.; Puritan-Bennet; Schuco Inc.; Omron Healthcare Inc.; DeVilbiss Health Care Inc; and Hospitak Inc.

5 Ultrasonic nebulizers deliver high medication output and are used by patients-suffering from severe asthma, or other severe respiratory related illnesses.

 The particles comprise a therapeutic or diagnostic agent. (therapeutic agents are sometimes referred to as drugs or pharmaceuticals. The
10 diagnostic agent referred to is typically a contrast agent such as an x-ray contrast agent but can also be other diagnostic materials.) The therapeutic or diagnostic agent exists as a discrete, crystalline phase. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as described in EPO 275,796.

15 The invention can be practiced with a wide variety of therapeutic or diagnostic agents. The therapeutic or diagnostic agent preferably is present in an essentially pure form. The therapeutic or diagnostic agent must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble" it is meant that the therapeutic or diagnostic agent has a solubility in the liquid dispersion medium
20 of less than about 10 mg/mL, and preferably of less than about 1 mg/mL. A preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which a therapeutic or diagnostic agent is poorly soluble and dispersible including, for example, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane and glycol. The pH
25 of the aqueous dispersion media can be adjusted by techniques known in the art.

 Suitable therapeutic or diagnostic agents can be selected from a variety of known classes of therapeutic or diagnostic agents including, for example, analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants,
30 antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media,
35 corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian

agents), haemostatics, immuriological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines. Preferred therapeutic or diagnostic agents include those intended for oral administration and intravenous administration. A description of these classes of therapeutic agents and diagnostic agents and a listing of species within each class can be found in Martindale, *The Extra Pharmacopoeia*, Twenty-ninth Edition, The Pharmaceutical Press, London, 1989.

10 The therapeutic or diagnostic agents are commercially available and/or can be prepared by techniques known in the art.

Preferred diagnostic agents include the x-ray imaging agent WIN-8883 (ethyl 3,5-diacetamido-2,4,6-triiodobenzoate) also known as the ethyl ester of diatrizoic acid (EEDA), WIN 67722, i.e., (6-ethoxy-6-oxohexyl-3,5-bis(acetamido)- 2,4,6-triiodobenzoate; ethyl-2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)butyrate (WIN 16318); ethyl diatrizoxyacetate (WIN 12901); ethyl 2-(3,5bis(acetamido)-2,4,6-triiodobenzoyloxy)propionate (WIN 16923); N-ethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy acetamide (WIN 65312); isopropyl 2(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy) acetamide (WIN 12855); diethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy malonate (WIN 67721); ethyl 2-(3,5bis(acetamido)-2,4,6-triiodobenzoyloxy) phenylacetate (WIN 67585); propanedioic acid, [[3,5-bis(acetylamino)2,4,5-triiodobenzoyl]oxy]-,bis(1-methyl)ester (WIN 68165); and benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-, 4-(ethyl-3-ethoxy-2-butenate) ester (WIN 68209). Suitable diagnostic agents are also disclosed in U.S. Patent No. 5,260,478; U.S. Patent No. 5,264,610; U.S. Patent No. 5,322,679 and U.S. Patent No. 5,300,739.

Preferred contrast agents include those which are expected to disintegrate relatively rapidly under physiological conditions, thus minimizing any particleassociated inflammatory response. Disintegration may result from enzymatic hydrolysis, solubilization of carboxylic acids at physiological pH, or other mechanisms. Thus, poorly soluble iodinated carboxylic acids such as iodipamide, diatrizoic acid, and metrizoic acid, along with hydrolytically labile iodinated species such as WIN 67721, WIN 12901, WIN 68165, and WIN 68209 or others may be preferred.

Surface Modifiers

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants.

5 Preferred surface modifiers include nonionic and ionic surfactants.

Representative examples of surface modifiers include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl
10 ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tween[™], polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose,
15 hydroxy propylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these surface modifiers are known pharmaceutical excipients and are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical
20 Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, poloxamers such as Pluronic[™] F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, and
25 polyxamines such as Tetronic[™] 908 (also known as Poloxamine[™] 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, dextran, lecithin, dialkylesters of sodium sulfosuccinic acid, such as Aerosol OTs[™], which is a dioctyl ester of sodium sulfosuccinic acid, available from American
30 Cyanimid, Duponol[™] P, which is a sodium lauryl sulfate, available from DuPont, Tritons[™] X-200, which is an alkyl aryl polyether sulfonate, available from Rohn and Haas, Tween[™] 20 and Tween[™] 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Specialty Chemicals; Carbowax[™] 3550 and 934, which are polyethylene glycols available from Union Carbide; Crodestas[™] F-110,
35 which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodestas[™] SL-40, which is available from Croda, Inc., and SA9OHCO,

which is $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4(CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include Tetronics™ 908, the Tweenss™, Plurionics™ F-68 and polyvinylpyrrolidone. Other useful surface modifiers 15 include:

- 5 decanoyl-N-methylglucamide;
 n-decyl β-D-glucopyranoside;
 n-decyl β-D-maltopyranoside;
 n-dodecyl β-D-glucopyranoside;
 n-dodecyl β-D-maltoside;
- 10 heptanoyl-N-methylglucamide;
 n-heptyl-β-D-glucopyranoside;
 n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside;
 nonanoyl-N-methylglucamide;
 n-nonyl β-D-glucopyranoside;
- 15 octanoyl-N-methylglucamide;
 n-octyl-β-D-glucopyranoside;
 octyl β-D-thioglucopyranoside; and the like.

Another useful surface modifier is tyloxapol (a nonionic liquid polymer of the alkyl aryl polyether alcohol type; also known as superinone or 20 triton). This surface modifier is commercially available and/or can be prepared by techniques known in the art.

Another preferred surface modifier is p-isononylphenoxypoly(glycidol) also known as Olin-LOG™ or Surfactant 10-G, is commercially available as LOG™ from Olin Chemicals, Stamford, Connecticut.

25

Non-Ionic Surface Modifiers

Preferred surface modifiers can be selected from known non-ionic surfactants, including the poloxamines such as Tetronic™ 908 (also known as Poloxamine™ 908), which is a tetrafunctional block copolymer derived from 30 sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, or Tetronic™ 1508 (T-1508), or a polymer of the alkyl aryl polyether alcohol type, such as tyloxapol.

The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two-or more surface modifiers can be 35 used in combination.

Tyloxapol

Tyloxapol (4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde) is a preferred surface modifier and is a nonionic liquid polymer of the alkyl aryl polyether alcohol type. Tyloxapol, also known as "Superinone", is disclosed as useful as a nonionic surface active agent in a lung surfactant composition in U.S. Patent No. 4,826,821 and as a stabilizing agent for 2-dimethylaminoethyl 4-n-butylaminobenzoate in U.S. Patent No. 3,272,700.

Tyloxapol may be associated with the nanoparticles and may function as a surface modifier, as a stabilizer, and/or as a dispersant. Alternatively, the tyloxapol may serve other purposes. Tyloxapol may serve all three functions. The tyloxapol may serve as a stabilizer and/or a dispersant, whereas another compound acts as a surface modifier.

Auxiliary Surface Modifiers

Particularly preferred auxiliary surface modifiers are those which impart resistance to particle aggregation during sterilization and include dioctylsulfosuccinate (DOSS), polyethylene glycol, glycerol, sodium dodecyl sulfate, dodecyl trimethyl ammonium bromide and a charged phospholipid such as dimyristoyl phosphatidyl glycerol. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Block Copolymer Surface Modifiers

One preferred surface modifier is a block copolymer linked to at least one anionic group. The polymers contain at least one, and preferably two, three, four or more anionic groups per molecule.

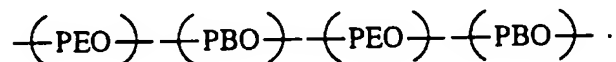
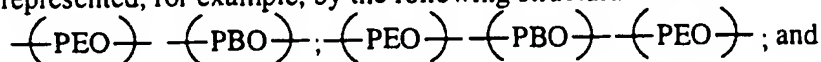
Preferred anionic groups include sulfate, sulfonate, phosphonate, phosphate and carboxylate groups. The anionic groups are covalently attached to the nonionic block copolymer. The nonionic sulfated polymeric surfactant has a molecular weight of 1,000-50,000, preferably 2,000-40,000 and more preferably 3,000- 30,000. In preferred embodiments, the polymer comprises at least about 50%, and more preferably, at least about 60% by weight of hydrophilic units, e.g., alkylene oxide units. The reason for this is that the presence of a major weight proportion of hydrophilic units confers aqueous solubility to the polymer.

A preferred class of block copolymers useful as surface modifiers herein includes sulfated block copolymers of ethylene oxide and propylene oxide.

These block copolymers in an unsulfated form are commercially available as Pluronics™. Specific examples of the unsulfated block copolymers include F68, F108 and F127.

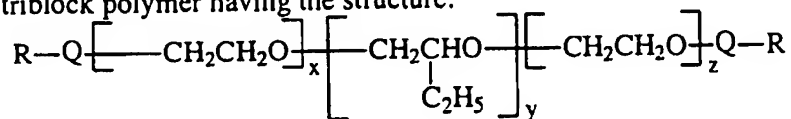
Another preferred class of block copolymers useful herein include tetrafunctional block copolymers derived from sequential addition of ethylene oxide and propylene oxide to ethylene diamine. These polymers, in an unsulfated form, are commercially available as Tetronics™.

Another preferred class of surface modifiers contain at least one polyethylene oxide (PEO) block as the hydrophilic portion of the molecule and at least one polybutylene oxide (PBO) block as the hydrophobic portion. Particularly preferred surface modifiers of this class are diblock, triblock, and higher block copolymers of ethylene oxide and butylene oxide, such as are represented, for example, by the following structural formula:



The block copolymers useful herein are known compounds and/or can be readily prepared by techniques well known in the art.

Highly preferred surface modifiers include triblock copolymers of the $\text{-(PEO)- (PBO)- (PEO)-}$ having molecular weights of 3800 and 5000 which are commercially available from Dow Chemical, Midland, Michigan, and are referred to as B20-3800 and B20-5000. These surface modifiers contain about 80% by weight PEO. In a preferred embodiment, the surface modifier is a triblock polymer having the structure:



Q is an anionic group

wherein R is H or a metal cation such as Na⁺, K⁺ and the like,
 x is 15-700,
 Y is 5-200 and
 z is 15-700. 30

Grinding

The described particles can be prepared in a method comprising the steps of dispersing a therapeutic or diagnostic agent in a liquid dispersion medium

and applying mechanical means in the presence of grinding media to reduce the particle size of the therapeutic or diagnostic agent to an effective average particle size of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

The therapeutic or diagnostic agent selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse therapeutic or diagnostic agent selected be less than about 10 mm as determined by sieve analysis. If the coarse particle size of the therapeutic or diagnostic agent is greater than about 100 mm, then it is preferred that the particles of the therapeutic or diagnostic agent be reduced in size to less than 100 mm using a conventional milling method such as airjet or fragmentation milling.

The coarse therapeutic or diagnostic agent selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the therapeutic or diagnostic agent in the liquid medium can vary from about 0.1 - 60%, and preferably is from 5 - 30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90%, and preferably is 1-75%, more preferably 20-60%, by weight based on the total combined weight of the therapeutic or diagnostic agent and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the dispersion to less than 1000 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the therapeutic or diagnostic agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates-visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition. Alternatively, the therapeutic or diagnostic agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected

to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the therapeutic or diagnostic agent conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

Preparation Conditions

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the therapeutic or diagnostic agent. Processing temperatures of less than about 30 - 40 C are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Control of the temperature, e.g., by jacketing or immersion of the milling chamber in ice water are contemplated. Processing pressures from about 1 psi (0.07 kg/cm²) up to about 50 psi (3.5 kg/cm²) are contemplated. Processing pressures from about 10 psi (0.7 kg/cm²) to about 20 psi 1.4 kg/cm²

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20 - 80 kHz for a time of about 1 to 120

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seconds.

After attrition is completed, the grinding media is separated from the milled particulate product (in either a dry or liquid dispersion form) using conventional separation techniques, such as by filtration, sieving through a mesh screen, and the like.

Grinding Media

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. We have found that zirconium oxide, such as 95% ZrO₂ stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. However, other media, such as stainless steel, titania, alumina, and 95% ZrO₂ stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 3 g/cm³.

Polymeric Grinding Media

The grinding media can comprise particles, preferably substantially spherical in shape, e.g., beads, consisting essentially of polymeric resin. Alternatively, the grinding media can comprise particles comprising a core having a coating of the polymeric resin adhered thereon.

In general, polymeric resins suitable for use herein are chemically and physically inert, substantially free of metals, solvent and monomers, and of sufficient hardness and friability to enable them to avoid being chipped or crushed during grinding. Suitable polymeric resins include crosslinked polystyrenes, such as polystyrene crosslinked with divinylbenzene, styrene copolymers, polycarbonates, polyacetals, such as DelrinTM, vinyl chloride polymers and copolymers, polyurethanes, polyamides, poly(tetrafluoroethylenes), e.g., TeflonTM, and other fluoropolymers, high density polyethylenes, polypropylenes, cellulose ethers and esters such as cellulose acetate, polyhydroxymethacrylate, polyhydroxyethyl acrylate, silicone containing polymers such as polysiloxanes and the like. The polymer can be biodegradable. Exemplary biodegradable

polymers include poly(lactides), poly(glycolide) copolymers of lactides and glycolide, polyanhydrides, poly(hydroxyethyl methacrylate), poly(imino carbonates), poly(N-acylhydroxyproline)esters, poly(N-palmitoyl hydroxyproline) esters, ethylene-vinyl acetate copolymers, poly(orthoesters), poly(caprolactones),
5 and poly(phosphazenes). In the case of biodegradable polymers, contamination from the media itself advantageously can metabolize in vivo into biologically acceptable products which can be eliminated from the body.

The polymeric resin can have a density from 0.8 to 3.0 g/cm³. Higher density resins are preferred inasmuch as it is believed that these provide
10 more efficient particle size reduction.

The media can range in size from about 0.1 to 3 mm. For fine grinding, the particles preferably are from 0.2 to 2 mm, more preferably, 0.25 to 1 mm in size.

In a particularly preferred method, a therapeutic or diagnostic agent
15 is prepared in the form of submicron particles by grinding the agent in the presence of a grinding media having a mean particle size of less than about 75 microns.

The core material of the grinding media preferably can be selected from materials known to be useful as grinding media when fabricated as spheres
20 or particles. Suitable core materials include zirconium oxides (such as 95% zirconium oxide stabilized with magnesia or yttrium), zirconium silicate, glass, stainless steel, titania, alumina, ferrite and the like. Preferred core materials have a density greater than about 2.5 g/cm³. The selection of high density core materials is believed to facilitate efficient particle size reduction.

Useful thicknesses of the polymer coating on the core are believed
25 to range from about 1 to about 500 microns, although other thicknesses outside this range may be useful in some applications. The thickness of the polymer coating preferably is less than the diameter of the core.

The cores can be coated with the polymeric resin by techniques
30 known in the art. Suitable techniques include spray coating, fluidized bed coating, and melt coating. Adhesion promoting or tie layers can optionally be provided to improve the adhesion between the core material and the resin coating. The adhesion of the polymer coating to the core material can be enhanced by treating the core material to adhesion promoting procedures, such as roughening of the
35 core surface, corona discharge treatment, and the like.

Continuous Grinding

In a preferred grinding process, the particles are made continuously rather than in a batch mode. The continuous method comprises the steps of continuously introducing the therapeutic or diagnostic agent and rigid grinding media into a milling chamber, contacting the agent with the grinding media while in the chamber to reduce the particle size of the agent, continuously removing the agent and the grinding media from the milling chamber, and thereafter separating the agent from the grinding media.

The therapeutic or diagnostic agent and the grinding media are continuously removed from the milling chamber. Thereafter, the grinding media is separated from the milled particulate agent (in either a dry or liquid dispersion form) using conventional separation techniques, in a secondary process such as by simple filtration, sieving through a mesh filter or screen, and the like. Other separation techniques such as centrifugation may also be employed.

In a preferred embodiment, the agent and grinding media are recirculated through the milling chamber. Examples of suitable means to effect such recirculation include conventional pumps such as peristaltic pumps, diaphragm pumps, piston pumps, centrifugal pumps and other positive displacement pumps which do not use sufficiently close tolerances to damage the grinding media. Peristaltic pumps are generally preferred.

Another variation of the continuous process includes the use of mixed media sizes. For example, larger media may be employed in a conventional manner where such media is restricted to the milling chamber. Smaller grinding media may be continuously recirculated through the system and permitted to pass through the agitated bed of larger grinding media. In this embodiment, the smaller media is preferably between about 1 and 300 μ m in mean particle size and the larger grinding media is between about 300 and 1000 μ m in mean particle size.

Precipitation Method

Another method of forming the desired nanoparticle dispersion is by microprecipitation. This is a method of preparing stable dispersions of therapeutic and diagnostic agents in the presence of a surface modifying and colloid stability enhancing surface active agent free of trace of any toxic solvents or solubilized heavy metal impurities by the following procedural steps:

1. Dissolving the therapeutic or diagnostic agent in aqueous

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base with stirring,

2. Adding above #1 formulation with stirring to a surface active surfactant (or surface modifiers) solution to form a clear solution, and

3. Neutralizing above formulation #2 with stirring with an appropriate acid solution. The procedure can be followed by:

4. Removal of formed salt by dialysis or diafiltration and

5. Concentration of dispersion by conventional means.

This microprecipitation process produces dispersion of therapeutic or diagnostic agents with Z-average particle diameter less than 400 nm (as measured by photon correlation spectroscopy) that are stable in particle size upon keeping under room temperature or refrigerated conditions. Such dispersions also demonstrate limited particle size growth upon autoclave-decontamination conditions used for standard blood-pool pharmaceutical agents.

Step 3 can be carried out in semicontinuous, continuous batch, or continuous methods at constant flow rates of the reacting components in computercontrolled reactors or in tubular reactors where reaction pH can be kept constant using pH-stat systems. Advantages of such modifications are that they provide cheaper manufacturing procedures for large-scale production of nanoparticulate dispersion systems.

Additional surface modifier may be added to the dispersion after precipitation. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20-80 kHz for a time of about 1 to 120 seconds.

In a preferred embodiment, the above procedure is followed with step 4 which comprises removing the formed salts by diafiltration or dialysis. This is done in the case of dialysis by standard dialysis equipment and by diafiltration using standard diafiltration equipment known in the art. Preferably, the final step is concentration to a desired concentration of the agent dispersion. This is done either by diafiltration or evaporation using standard equipment known in this art.

An advantage of microprecipitation is that unlike milled dispersion, the final product is free of heavy metal contaminants arising from the milling media that must be removed due to their toxicity before product is formulated.

A further advantage of the microprecipitation method is that unlike

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solvent precipitation, the final product is free of any trace of trace solvents that may be toxic and must be removed by expensive treatments prior to final product formulation.

In another preferred embodiment of the microprecipitation process, a crystal growth modifier is used. A crystal growth modifier is defined as a compound that in the co-precipitation process incorporates into the crystal structure of the microprecipitated crystals of the pharmaceutical agent, thereby hindering growth or enlargement of the microcrystalline precipitate, by the so called Ostwald ripening process. A crystal growth modifier (or a CGM) is a chemical that is at least 75% identical in chemical structure to the pharmaceutical agent. By "identical" is meant that the structures are identical atom for atom and their connectivity. Structural identity is characterized as having 75% of the chemical structure, on a molecular weight basis, identical to the therapeutic or diagnostic agent. The remaining 25% of the structure may be absent or replaced by different chemical structure in the CGM. The crystal growth modifier is dissolved in step #1 with the therapeutic or diagnostic agent.

Particle Size

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. When photon correlation spectroscopy (PCS) is used as the method of particle sizing the average particle diameter is the Z-average particle diameter known to those skilled in the art. By "an effective average particle size of less than about 1000 nm" it is meant that at least 90% of the particles have a weight average particle size of less than about 1000 nm when measured by the above-noted techniques. In preferred embodiments, the effective average particle size is less than about 400 nm and more preferably less than about 300nm. In some embodiments, an effective average particle size of less than about 100 nm has been achieved. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e.g., 1000 nm. In particularly preferred embodiments essentially all of the particles have a size less than 1000 nm. In some embodiments, essentially all of the particles have a size less than 400 nm.

Ratios

The relative amount of therapeutic or diagnostic agent and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular therapeutic or diagnostic agent and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the hydrophilic lipophilic balance (HLB) of the stabilizer, the melting point of the stabilizer, its water solubility, the surface tension of water solutions of the stabilizer, etc. The surface modifier preferably is present in an amount of about 0.1-10 mg per square meter surface area of the therapeutic or diagnostic agent. The surface modifier can be present in an amount of 0.1-90%, preferably 20-60% by weight based on the total weight of the dry particle.

Diagnosis

A method for diagnostic imaging for use in medical procedures in accordance with this invention comprises administering to the body of a test subject in need of a diagnostic image an effective contrast producing amount of the diagnostic image contrast composition. In addition to human patients, the test subject can include mammalian species such as rabbits, dogs, cats, monkeys, sheep, pigs, horses, bovine animals and the like. Thereafter, at least a portion of the body containing the administered contrast agent is exposed to x-rays or a magnetic field to produce an x-ray or magnetic resonance image pattern corresponding to the presence of the contrast agent. The image pattern can then be visualized.

Any x-ray visualization technique, preferably, a high contrast technique such as computed tomography, can be applied in a conventional manner. Alternatively, the image pattern can be observed directly on an x-ray sensitive phosphor screen-silver halide photographic film combination or by use of a storage phosphor screen.

Visualization with a magnetic resonance imaging system can be accomplished with commercially available magnetic imaging systems such as a General Electric 1.5 T Sigma imaging system [1H resonant frequency 63.9 megahertz (MHz)]. Commercially available magnetic resonance imaging systems are typically characterized by the magnetic field strength used, with a field strength of 2.0 Tesla as the current maximum and 0.2 Tesla as the current minimum. For a given field strength, each detected nucleus has a characteristic frequency. For example, at a field strength of 1.0 Tesla, the resonance frequency

for hydrogen is 42.57

10 MHz; for phosphorus-31 it is 17.24 MHz; and for sodium23 it is 11.26 Mhz.

A contrast effective amount of the diagnostic agent containing composition is that amount necessary to provide tissue visualization with, for
5 example, magnetic resonance imaging or x-ray imaging. Means for determining a contrast effective amount in a particular subject will depend, as is well known in the art, on the nature of the magnetically reactive material used, the mass of the subject being imaged, the sensitivity of the magnetic resonance or x-ray imaging system and the like.

10 After administration of the compositions, the subject mammal is maintained for a time period sufficient for the administered compositions to be distributed throughout the subject and enter the tissues of the mammal. Typically, a sufficient time period is from about 20 minutes to about 90 minutes and, preferably from about 20 minutes to about 60 minutes. The following examples
15 are presented for a further understanding of the invention.

Example 1 Using the Therapeutic Agent Beclomethasone

Materials. Beclomethasone dipropionate (BDP) and polyvinyl alcohol (PVA) were obtained from Sigma Chemical Co. (St. Louis, MO) and used
20 as received. All other chemicals were analytical/reagent grade or better.

Nanoparticle Preparation and Characterization. Nanoparticles were prepared by media milling a suspension of 5% beclomethasone dipropionate in an aqueous solutions of PVA. Thus, the PVA was the surface modifier. The resulting particle size distribution was determined by dynamic light scattering.
25 The particle size distribution was periodically monitored throughout the course of the study.

Nebulization. A gas cylinder of compressed air was used as the source, which was equipped with a pressure regulator. Oxygen connecting tubing joined from the regulator to the Puritan-Bennet Raindrop nebulizer (Lenexa, KA).
30 One exit port of the T-connector of the nebulizer was blocked with a #2 rubber stopper. The other exit port was fitted with Tygon tubing (1/2" id). This in turn led initially to a calibrated flow meter from which the flow rate was set before each experiment. After calibration, the gas flow was stopped by shutting off the main cylinder valve. The flow meter was removed, and the nebulizer was
35 connected to a Y-tube with 24/40 joints by tubing (1/2" id, 6" length). The Y-tube was connected to the cascade impactor (Andersen Mark I, Andersen

Samplers Ind. Atlanta, GA) by a constructed stainless steel adapter consisting of a tapered side that fit within the 24/40 ground glass joint and a cylindrical section with rubber o-ring gasket that fit into the top of the cascade impactor. The air flow rate through the impactor was drawn by a vacuum pump and regulated by a calibrated flow meter to the recommended 28.3 L/min.

Preliminary studies indicated that pressures between 20 and 40 psig had little effect on either the performance of the nebulizer or the resulting aerosol size distribution. Thus, the pressure was kept constant at 40 psig. Studies of the effect of flow rate on nebulizer performance and aerosol size distribution were also conducted. As the flow rate was decreased from 5 to 2 L/min, aerosol particles had progressively larger mean aerodynamic diameter. At a flow rate 8 L/min, there was excessive foaming. Thus, all studies were conducted at a flow rate of 6 L/min.

Suspension and Nanoparticle Nebulization. Formulations for nebulization consisted of a 0.2% beclomethasone dipropionate dispersions with PVA. The nebulizers contained either a volume of 2 mL or 6 mL. Two concentrations of PVA were used which were prepared by diluting the original 5% (w/v) nanoparticle dispersion with a PVA solution having the same PVA concentration as the original dispersion concentration or with water. The nebulizer was filled, and aliquots of the solution were taken for subsequent determination of drug concentration. The weight was also determined. The nebulization process was initiated by opening the valve on the main gas cylinder, and the length of time until foaming or sputtering of the nebulizer was determined, and additional aliquots were taken for analysis. The fraction of mass exiting the nebulizer was calculated from the weight difference of the nebulizer before and after nebulization. This was coupled with the time required for nebulization of the dispersion to yield the mass output rate in terms of the milliliters of dispersion nebulized/unit time and the nebulizer output in terms of the volume of dispersion nebulized/liter of air were determined.

Aliquots taken from the nebulizer were diluted with 50% (v/v) ethanol in water, and the absorbance determined at 240 nm. With measurement of the absorbance of appropriate standards, the concentration of BDP was calculated. From the masses of the nebulizer before and after nebulization and the BDP concentrations, the fraction of BDP remaining in the nebulizer was calculated. The mass of BDP collected on the cascade impactor and the aerosol particle size distribution was determined by extracting the impactor stages with 10 mL of the

ethanol/water solution. Aliquots were taken and the absorbances and subsequent concentration were determined. The mass median aerodynamic diameter and geometric standard deviation of the particle distribution was obtained by plotting the cumulative mass on the stages of the impactor as a function of the log of the cut-off diameter. With the cumulative mass determined from the cascade
5 impactor and the initial amount of BDP placed in the nebulizer, the fraction of BDP reaching the impactor was calculated.

To assess the fractionation of the dispersion, the nanoparticles and suspensions were diluted with PVA solutions containing 0.1% sodium fluorescein. Nebulization was conducted as described above. Since fluorescein has significant
10 absorbance at both 490 and 240 nm while BDP has absorbance only at 240 nm, the absorbance of the diluted aliquots was determined at these two wavelengths. The concentration of fluorescein was determined from the absorbance at 490 nm and the measured absorptivity. In determining the concentrations of BDP, the
15 contribution from the absorbance of fluorescein at 240 nm was subtracted based on the absorbance determined at 490 and the correction for the differences in the absorptivity at these two wavelength.

Scanning Electron Microscopy. SEM was performed on nanoparticles after nebulization. Two dispersions were prepared containing 0.1
20 and 2.5% surfactant. These were placed in the nebulizer and 2 cm rectangular glass microscope slides were placed on every stage of the impactor. The glass slides were removed and sputtered 5 with platinum. Micrographs were obtained with a JEOL 840-II ElectroScan Environmental ESEM (Peabody, Mass.).

25 **RESULTS**

Nanoparticles of beclomethasone dipropionate in 2.5% polyvinyl alcohol had a particle size distribution of 0.26 ± 0.13 μ m. This size remained constant throughout the course of the study; neither was there any evidence of chemical instability. In addition, particle size of the diluted dispersions remained
30 constant for at least the duration of the experiment.

For nebulization, four formulations were tested. These are listed in Table I. The first was a suspension of raw drug substance BDP in 2.5% surfactant with a volume of 2 mL. The second was composed of a dispersion of nanoparticles thereby allowing direct comparison to the suspension formulation.
35 The third was also a colloidal dispersin, but the surfactant concentration was smaller at 0.1%. The fourth was similar to the third but contained a larger volume

of 6 mL.

In Table II, the results from the nebulization of the four formulations were given. The second column provides the mass output rate which was the rate at which the total mass of the dispersion exists the nebulizer.

5 Formulations I and II are similar as were formulations III and IV. The difference between these two sets of formulations is that I and II had a surfactant concentration of 2.5%, whereas III and IV had a surfactant concentration of 0.1%.

The third column reflects the total mass fraction of dispersion remaining in the nebulizer. The fraction of mass remaining was between 0.27 and 10 0.69 indicating considerable amount of material remained in the nebulizer. In addition, formulations I, II and III were similar, but formulation IV had a significantly lower mass fraction remaining in the nebulizer. Formulation IV is distinct from the others in that it contained an initial volume of 6 mL.

In the next column, the fraction of BDP remaining in the nebulizer is given. These fractions ranged from 0.29 to 0.89. In comparing the fractions 15 remaining, formulation I, which contained the suspension, had about 90% of BDP remain in the nebulizer. In contrast, formulation III which contained 0.1% surfactant, had a significantly lower fraction of BDP remain in the nebulizer. An even more dramatic drop in fraction remaining was observed with formulation IV 20 which had a low surfactant concentration as well as a larger volume.

It is also noteworthy to compare the fraction of BDP remaining relative to the fraction of total mass remaining in the nebulizer. With formulation I, there was a significantly greater fraction of BDP relative to the total mass remaining. Numerically this is also true for formulation II: however, there was 25 more variability in these measurements which had no statistical difference in the fractions remaining. In formulations III and IV, there was no difference.

The fraction of BDP reaching the nebulizer is also given in Table II. It is seen that only about 7% of the BDP presented as a suspension or raw drug substance reaches the impactor. In comparison, the use of nanoparticles led 30 to a significantly higher fraction reaching the impactor. These ranged from 0.17 to over 0.34. In formulations II and III which contained 2 mL of dispersion, about 18% of BDP reached the impactor. In the large volume formulation IV, almost 35% of BDP reached the impactor.

Finally, it is evident that the amount of BDP that was originally 35 placed in the nebulizer should equal the amount of BDP remaining in the nebulizer added to the amount of BDP on the impactor. Expressing the mass

balance in terms of fractions, the fraction of BDP remaining in the nebulizer plus the fraction of BDP on the impactor should equal unity. As can be deduced from the fractions given in Table II, this was only the case with formulation II. In other cases, there was a net loss of BDP. In particular, for formulation III, only 80% of BDP was accounted for, and in formulation IV, the percent accounted for dropped to about 60%.

It is evident when the fraction of BDP collected on the impactor stage is plotted as a function of the cut-off diameter of the stage that suspensions of raw drug substance have a distribution of particles with a larger size and its distribution is more polydisperse. The nanoparticles have particles size distributions with 80% of the particles being less than 2.5 μ m.

In Table III, the results from the fluorescein study are given. In comparing the mass exited, both formulations gave similar results of about 0.75. There was also no significant difference between the fractions of BDP and fluorescein remaining in the nebulizer. For the suspension, the fraction of BDP and fluorescein remaining were 88 and 89%, respectively. For the nanoparticles, the percents were 81 and 85 which are not statistically different from each other. In addition, there was no statistical difference in the fractions of BDP and fluorescein remaining in the nebulizer between formulations I and II. However, the fractions of BDP and fluorescein remaining are significantly greater than the fraction of total mass remaining for the suspension and nanoparticle formulations.

The fractions of BDP reaching the impactor were different between the two formulations. For the suspension, the fraction of fluorescein collected on the impactor was almost twice as high as the fraction of BDP. For the nanoparticles, the fraction of fluorescein was similar to that found with suspensions. The fraction of BDP collected on the impactor was much higher than observed with suspensions, but slightly less than that observed with fluorescein.

The final study was an examination of the particles after being subjected to the process of nebulization. Scanning electron microscopy was conducted of the nanoparticles deposited on the sixth stage of the impactor for the 2.5 and 0.1 μ m nanoparticles.

Table I
Formulation Components

Formulation	Form	[Surfactant]	Volume (mL)
I	Suspension	2.5%	1.85
II	Nanoparticle Dispersion	2.5%	1.85
III	Nanoparticle Dispersion	0.1%	1.85
IV	Nanoparticle Dispersion	0.1%	5.85

Formulation "I" is a comparative formulation not using nanoparticles.

5

Table II
Comparison of Nebulization Output Parameters as a Function of Formulate
Effect of Nebulization Process on Resulting Aerosol Production. Results are
expressed as the mean + standard deviation, n=3.

Formulation	Mass Output Rate (mg/sec)	Mass Fraction Remain.	BDP Fraction Remain	BDP Fraction Remain
I	2.73 ± 0.5	0.69 ± 0.036	0.89 ± 0.013	0.082 ± 0.012
II	2.61 ± 0.14	0.51 ± 0.15	0.768 ± 0.23	0.184 ± 0.47
III	4.99 ± 0.31	0.67 ± 0.006	0.618 ± 0.025	0.174 ± 0.019
IV	4.35 ± 0.65	0.27 ± 0.015	0.289 ± 0.039	0.345 ± 0.15

Table III
Comparison of Nebulization of Nanoparticle Dispersions and Suspensions of
BDP Containing a Solution of Fluorescein

Formulation	Mass Fraction Remaining	BDP Fraction Remaining	Fluorescein Fraction Remaining	BDP Fraction on Impactor	Fluorescein Fraction On Impactor
Suspension	0.76 ± 0.06	0.88 ± 0.046	0.89 ± 0.13	0.067 ± 0.02	0.122 ± 0.033
Nanoparticles	0.74 ± 0.17	0.81 ± 0.088	0.85 ± 0.065	0.11 ± 0.016	0.143 ± 0.020

5 Example 2 Using a Contrast Agent

In this example, a suspension of WIN 68209 (30%) in aqueous F108 surfactant (6%) was prepared by conventional roller milling techniques (jar mill, zirconium silicate beads, 7 days milling time). The mean particle size of the resultant distribution was 196 nm. The formulation was administered to an anesthetized rabbit as follows: Several mL of formulation was placed in an ultrasonic nebulizer (DeVilbiss AeroSonic (TM)) which was connected in-line with a mechanical ventilator, terminating in a suitable endotracheal tube. The rabbit was then intubated and administered the nebulized formulation for several minutes. Subsequent computed tomography (CT) scans of the rabbit's pulmonary region showed the presence of radiopaque contrast agent in the region.

The invention has been described with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

Claims:

1. An aerosol comprising droplets of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof.
5
2. An aerosol according to claim 1 wherein said therapeutic agent is beclomethasone dipropionate.
- 10 3. An aerosol according to claim 1 wherein said diagnostic agent is benzoic acid, 3,5-bis(acetylamino)2,4,6-triiodo-, 4-(ethyl-3-ethoxy-2-butenate) ester (WIN 68209).
- 15 4. A method for forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof, said method comprising the steps of:
 - a) providing a suspension of said nanoparticles; and
 - b) nebulizing said suspension so as to form an aerosol.
20
5. A method of treating a mammal comprising the steps of:
 - a) forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic agent particles having a surface modifier on the surface thereof;
 - 25 b) administering said aerosol to the respiratory system of said mammal.
6. A method according to claim 5 wherein said aerosol is administered in a manner such that it reaches the lung.
30
7. A method according to claim 6 wherein said nanoparticles contain beclomethazone.
8. A method of diagnosing a mammal, said method comprising the
35 steps of:
 - a) forming an aerosol of an aqueous dispersion of

nanoparticles, said nanoparticles comprising insoluble diagnostic imaging agent particles having a surface modifier on the surface thereof; and

b) administering said aerosol to the respiratory system of said mammal; and

5 c) imaging said imaging agent in said respiratory system.

9. A method according to claim 8 wherein said diagnostic imaging agent is benzoic acid, 3,5bis(acetylamino)-2,4,6-triiodo-, 4-(ethyl-3-ethoxy-2butenoate) ester (WIN 68209).

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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US CL :424/45, 46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/45, 46

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,145,684 (LIVERSIDGE ET AL.) 08 September 1992, see entire document.	1-9
Y	WO 92/08446 (GALAXO GROUP LTD.) 29 May 1992, see entire document.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

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